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QUERCETIN AND DERIVATIVES AS BUTYRYLCHOLINESTERASE INHIBITORS

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INTRODUCTION

Alzheimer's disease is a neurogenerative disease which is caused by many factors. According to the cholinergic hypothesis, the reduction of acetylcholine (ACh) in the brain is one of major cause. Therefore, therapeutic goal of drug development is in order to search compounds which have anti-cholinesterase activity. Recently, some scientific studies suggest that in normal people acetylcholinesterase (AChE) plays major role to hydrolyze the ACh neurotransmitter while butyrylcholinesterase (BChE) plays minor role. In contrary, Alzheimer's BChE level increase dramatically with severity and become more important while AChE level can decline up to 85-90%. For these reasons, BChE could be a promising target for Alzheimer's disease therapeutic strategy.

Quercetin is a natural compound which classified as a flavonoid. It is commonly found in fruits and vegetables such as apples, onions, grapes or citrus fruits. Quercetin has shown the antioxidant, anti-AChE as well as anti-BChE activities. Therefore, in the present study, we were interested in testing whether quercetin derivatives were able to inhibit BChE and in delineating the possible structure-activity relationships. The information from this study would be useful for further development of Alzheimer's drug.

MATERIALS AND METHODS

***Methylation and ethylation*:**

Quercetin 1 equiv. was dissolved in DMF as less as possible. Then, 5.5 equiv. of methyl iodide or ethyl bromide were added. The potassium carbonate was introduced into the reaction flask, mixed together and stirred at room temperature for one week. The products were checked and further purified by column chromatography. Structure elucidation was performed by using UV, IR, and NMR.

***Alkaline treatment*:**

Quercetin was react with excess NaOH, mixed together and stirred with heat at 70-80°C for two days. Structure determination was achieved after the purification step.

***Anti-butyrylcholinesterase Activity testing*:**

The method of Ellman was modified and used for the assessment of the anti-BChE activity. Test samples and other chemicals were freshly prepared. Each sample was dissolved in DMSO to final concentration at 100 mcg/ml. Briefly, sodium phosphate buffer (pH 8.0), DTNB, test solution and butyrylthiocholine solution were added into 96-well microplate and incubated for 5 min at 37°C. Then, the reaction was initiated by the addition of BChE. After 20 min incubation, absorbance was measured at wavelength 405 nm. The percentage of enzyme inhibition was calculated.

RESULTS AND DISCUSSION

***Methylation and ethylation*:**

The alkylation of quercetin with alkyl halide; methyl iodide and ethyl bromide, gave a mixture of *O*-alkylated products. When quercetin was treated with ethyl bromide and K_2CO_3, two compounds, A and B, were obtained in 33.87 and 32.17% yield, respectively. Similarly, methylation of quercetin using methyl iodide and K_2CO_3 gave two compounds, C and D, in the %yield of 74.15 and 21.84, respectively. The structure determination of these compounds was successively identified.

***Alkaline treatment*:**

The alkaline treatment of quercetin in excess NaOH resulted in the soluble compound E. The identification of this compound was studied depending on spectroscopic data.

Identification:

UV spectral data was used to represent the characteristic of flavonoid compounds. The absorbance peaks were presented at two regions; 370-380 nm. and 250-260 nm. as shown in figure 1.

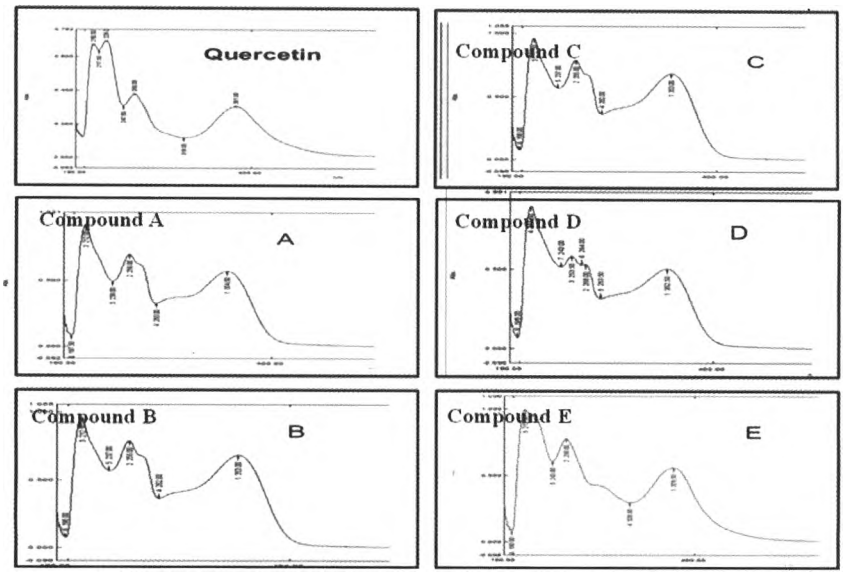


Figure 1: UV spectrum of quercetin and derivatives

IR spectral data was used to represent the functional group. As figure 2, all compounds contained O-H (broad band, 3600-3300 cm^{-1}) and C=C- (1650-1450 cm^{-1}) bands.

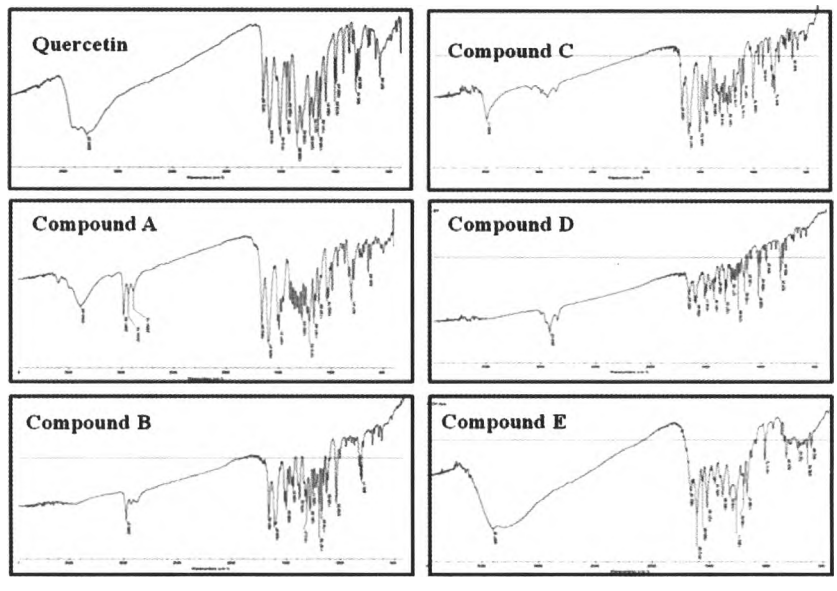
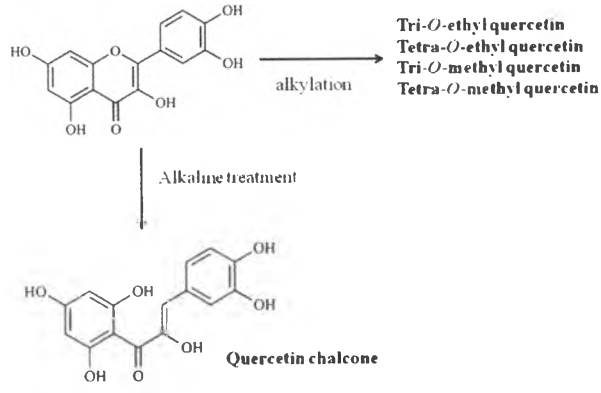


Figure 2: IR spectrum of quercetin and derivatives

NMR spectroscopy has been employed for the structural examination of all compounds. The ^{1}H NMR spectra of quercetin and its derivatives were shown in figure 3. Analyses of the ^{1}H NMR spectrum of compound A indicated the presence of three ethoxyl groups in the molecule due to the integration of methylene and methyl protons at the δ 4.10-4.32 and 1.50-.175 ppm, respectively. Thus, compound A was characterized as tri-*O*-ethyl quercetin. In the similar way, the NMR spectral data of compound B supported the four ethoxyl groups in comparison to quercetin. Therefore, compound B was identified as tetra-*O*-ethyl quercetin.

During methylation of quercetin, tri-*O*-methyl quercetin and tetra-*O*-methyl quercetin were obtained. The ^{1}H NMR spectrum of compound C showed three methoxyl protons at the δ 3.75-4.10 ppm., while four methoxyl protons were substituted in compound D. However, the position of alkyl substituted groups in compound A, B, C and D have to be further analyzed.

In the alkaline treatment reaction, ring C of quercetin was cleaved and produced an additional hydroxyl and olefmic protons. The ^{1}H NMR spectrum of compound E showed the olifenic proton at the δ 5.62 ppm. Hence, compound E was established as quercetin chalcone. All derivatives were shown in Scheme 1.



Scheme 1 Quercetin and derivatives

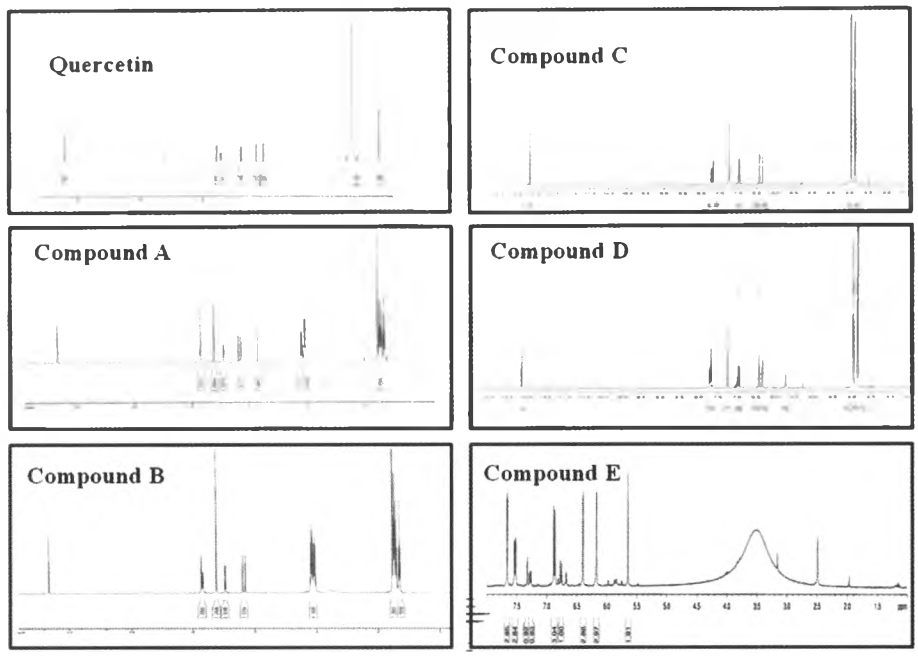


Figure 3: ^{1}H NMR spectrum of quercetin and derivatives

***Butyrylcholinesterase inhibitory activity testing*:**

Quercetin and derivatives were examined for their BChE inhibitory activity using modified Ellman method. Among them, only quercetin was found to inhibit enzyme activity with 56.36% inhibition at the concentration of 100 mcg/ml. While the other compounds showed inhibition toward this enzyme below 50%. However, quercetin chalcone was the most active derivative with 24.43% inhibition. Interesting, the results showed that the derivatives containing three alkyl substitutions exhibited more % inhibition than four alkyl substitutions as shown in Figure 4.

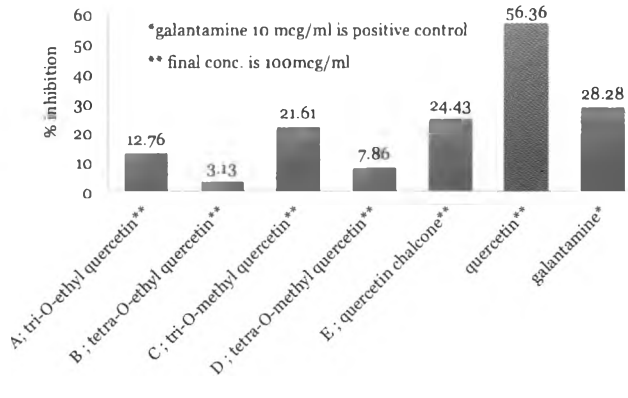


Figure 4: Anti-BChE activity of quercetin and derivatives

CONCLUSION

In summary, five derivatives, tri-*O*-methyl quercetin, tetra-*O*-methyl quercetin, tri-*O*-ethyl quercetin, tetra-*O*-ethyl quercetin, and quercetin chalcone, were prepared and evaluated for their potency. The most potent butyrylcholinesterase inhibitor is quercetin, followed by quercetin chalcone and tri-*O*-methyl quercetin, respectively. Further studies are being investigated.

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